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SCANNING ELECTRON MICROSCOPY OF EGGS OF *OCHLEROTATUS* (*PROTOMACLEAYA*) *TERRENS* WALKER

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ABSTRACT. Observations on the morphological ultrastructure of eggs of *Ochlerotatus* (*Protomacleaya*) *terrens* (Diptera: Culicidae) were conducted by using scanning electron microscopy. Morphometry of the principal structures was obtained with the aid of Semafore analysis software. Eggs of *Oc. terrens* from females caught in the Biological Reserve of Tinguá, State of Rio de Janeiro, Brazil, were utilized. The eggs presented elliptical outlines, with a length of approximately 649.0 μm and width of 168.7 μm. The egg index (length to width ratio) was 3.85 μm. The exochorion had hexagonal and sometimes pentagonal ornamentation. Inside the chorionic cells were small, well-distributed tubercles with a large range of sizes. The micropylar apparatus, located in the anterior region of the egg, presented a collar with a poorly visible frame, with borders of indeterminate extent and margins without a defined transition area, and a thickness of approximately 1.8 μm. The micropyle was plugged.

KEY WORDS *Ochlerotatus, Protomacleaya,* Culicidae, eggs, ultrastructure

INTRODUCTION

Reinert (2000) proposed a new composition for the genus *Aedes,* dividing it into 2 taxa, *Aedes* and *Ochlerotatus,* based on the consistency of primary characteristics in the female and male genitalia. This proposal elevated the subgenus *Ochlerotatus* to genus and reclassified other subgenera. With this new classification of the genera, the nomenclature of *Aedes* (*Finlaya*) *terrens* changed to *Ochlerotatus* (*Protomacleaya*) *terrens* Walker, 1856.

Ochlerotatus terrens is a strictly forest-dwelling species from the crowns of trees. It is a diurnal mosquito (Guimarães et al. 1985). The breeding sites for the species include tree holes and the internodes of bamboo plants. This culicid is distributed from Panama to southern South America, and has been found in Argentina, Brazil, Colombia, French Guiana, Paraguay, Suriname, and Venezuela (Walter Reed Biosystematics Unit 2001). Matsuo et al. (1974) described and illustrated the eggs of 4 species of *Aedes* from the subgenus *Finlaya,* from the Palaearctic region, by means of scanning electron microscopy (SEM), based on the reticulum and outline patterns.

The present study describes the eggs of *Oc. terrens* by using SEM and conducts morphometric analysis on the principal structures.

MATERIALS AND METHODS

Eggs of *Oc. terrens* were obtained from females caught in the Biological Reserve of Tinguá, which is located within the municipality of Nova Iguaçu, state of Rio de Janeiro, at the latitude of 22°28′–22°39′S and longitude of 43°13′–43°34′W. The mosquitoes were caught in Centers for Disease Control automatic CO_2-based traps and transported to the laboratory on the same day. Females in perfect condition were individually isolated in flat-bottomed glass tubes measuring 25 mm in diameter and 50 mm in height that, at the bottom, contained a piece of cotton wool that was moistened with water and covered with filter paper. This had the function of serving as a substrate for oviposition (Bates and Roca-Garcia 1945). Thirty eggs were collected from 20 females. Eleven of these eggs were submitted for morphometrical analysis. The specimens were identified by associating with male specimens obtained from the same egg batches and utilizing the key published by Arnell (1973).

Immediately after oviposition, the eggs were taken from the filter paper by using a fine artist's paintbrush (no. 2). The eggs were then fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, both in a 0.1 M, pH 7.2, sodium cacodylate buffer. After washing in the same buffer, the eggs were dehydrated in a series of increasing ethanol concentrations and submitted to the critical point drying method, using superdry CO_2 (Hayat 1970). After this, they were placed on metallic supports, covered with gold, and observed with a Jeol 5310 scanning electron microscope (Akishima, Tokyo, Japan).

The measurements were made directly from the images obtained, with the aid of Semafore analysis software (JEOL [Skandinoviska] AB, Soppentuma, Sweden) coupled to the microscope. The following parameters were utilized: total length, total width, thickness of the micropylar collar, and diameter and circumference of the chorionic cells. Only the calculated means have been cited, followed by standard deviations ($\bar{x} \pm$ SD). The terminology utilized for the description of the eggs follows that of Harbach and Knight (1980).

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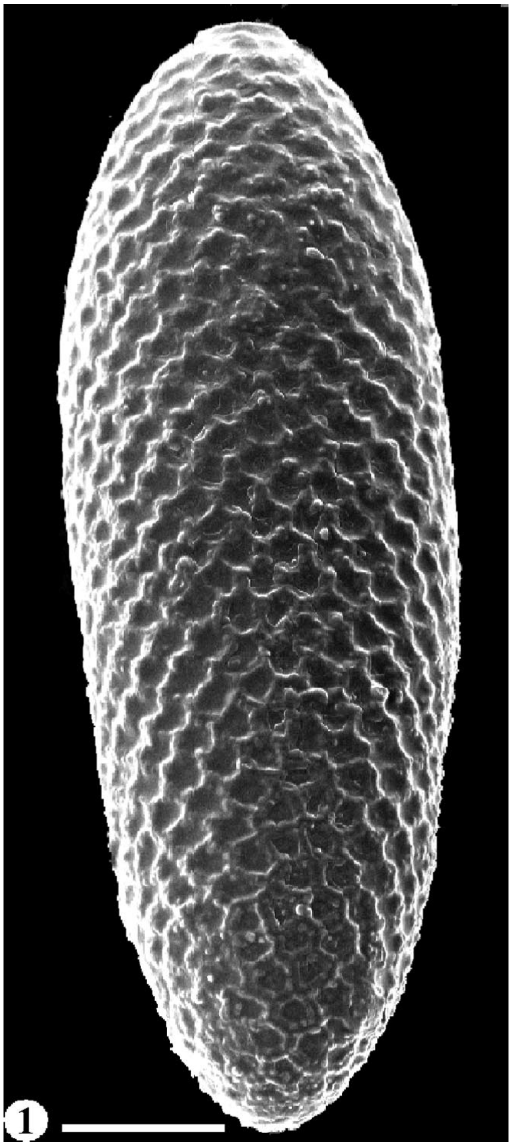

Fig. 1. Ventral (upper) view, anterior end at top of entire egg of *Ochlerotatus terrens.*

RESULTS

The eggs were elliptical in outline, with a length of approximately 649.0 μm and a width of 168.7 μm at the extremities (n = 10). The anterior region measured 58.0 μm at the level of the micropyle and 46.8 μm in the posterior region, with an egg index (length to width ratio) of 3.85 μm (Fig. 1).

Regular chorionic cells with hexagonal and sometimes pentagonal ornamentation were observed on the ventral surface. Each chorionic cell presented a thick, raised external chorionic reticulum, with regular borders (Fig. 2), a longitudinal diameter ($\bar{x} \pm$ SD) of 13.2 ± 1.4 μm (n = 10), and a circumference of 106.4 μm. Small, rounded tubercles that were well distributed occurred inside these ventral chorionic cells but were of variable size, from 0.58 to 1.50 μm in diameter.

On the dorsal surface, 2 types of tubercles were present in the chorionic cells, especially near the region of adhesion to the substrate. At the anterior portion of the egg near the micropyle, elongated fusiform tubercles, approximately 4.0 μm in height and 2.4 μm in diameter, were found inside the chorionic cells (Fig. 3). These tubercles presented a very regular pattern and were distributed symmetrically throughout the cell. In the midregion of the egg, the chorionic reticulum presented smaller tubercles inside each cell, whereas on the periphery of the cell, larger tubercles were observed, sometimes fused at the vertices. The external chorionic reticulum presented a porous appearance across the dorsal surface, and its thickness ranged from 0.7 to 3.4 μm.

The micropylar apparatus, located in the anterior area of the egg, was formed by a nonprominent collar with borders that were not well defined. Centrally, the micropylar disk had a diameter of approximately 19.9 μm. It was not possible to delimit the diameter of the micropylar orifice. The micropyle was plugged (Fig. 4).

DISCUSSION

In the present work the dorsal and ventral surfaces of eggs of *Ochlerotatus* showed significant differences in the shape and size of the tubercles present in the chorionic cells. Linley et al. (1991), working on eggs of *Aedes notoscriptus* Skuse, drew attention to the fact that important information could be lost through incomplete examination of the eggs. In fact, the importance of examining both the dorsal surface and the structure of the lateral surface of culicid eggs was emphasized and the present study examined both surfaces, which showed differences in tubercle size and shapes. The results of the present study support this recommendation.

Linley and Chadee (1991) demonstrated that eggs of *Haemagogus equinus* Theobald and *Hg. Janthinomys* Dyar contained highly developed filaments. These authors showed that the presence of such structures could improve the adhesion of the

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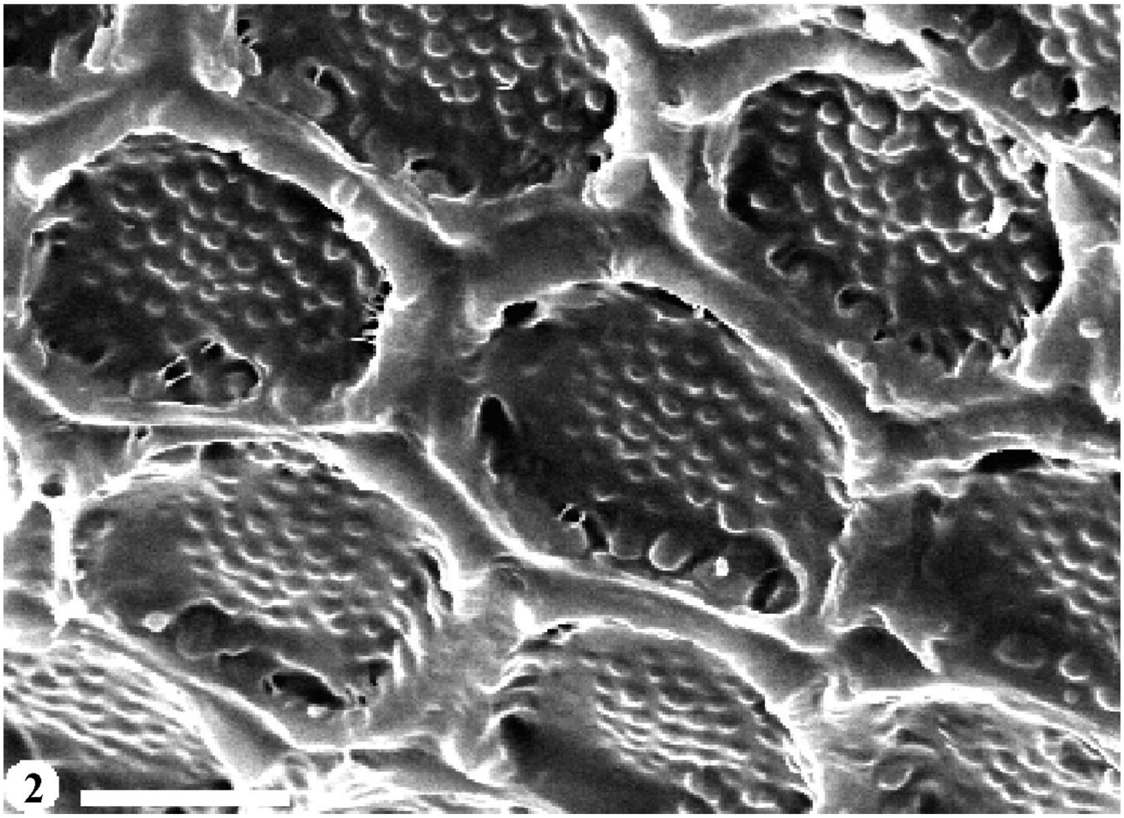
Fig. 2. Typical ornamentation of outer chorionic reticulum showing small tubercles in the center of a chorionic cell of the ventral surface of the egg.

Fig. 3. Dorsal surface of the anterior portion of the egg showing elongated fusiform tubercles inside the chorionic cells.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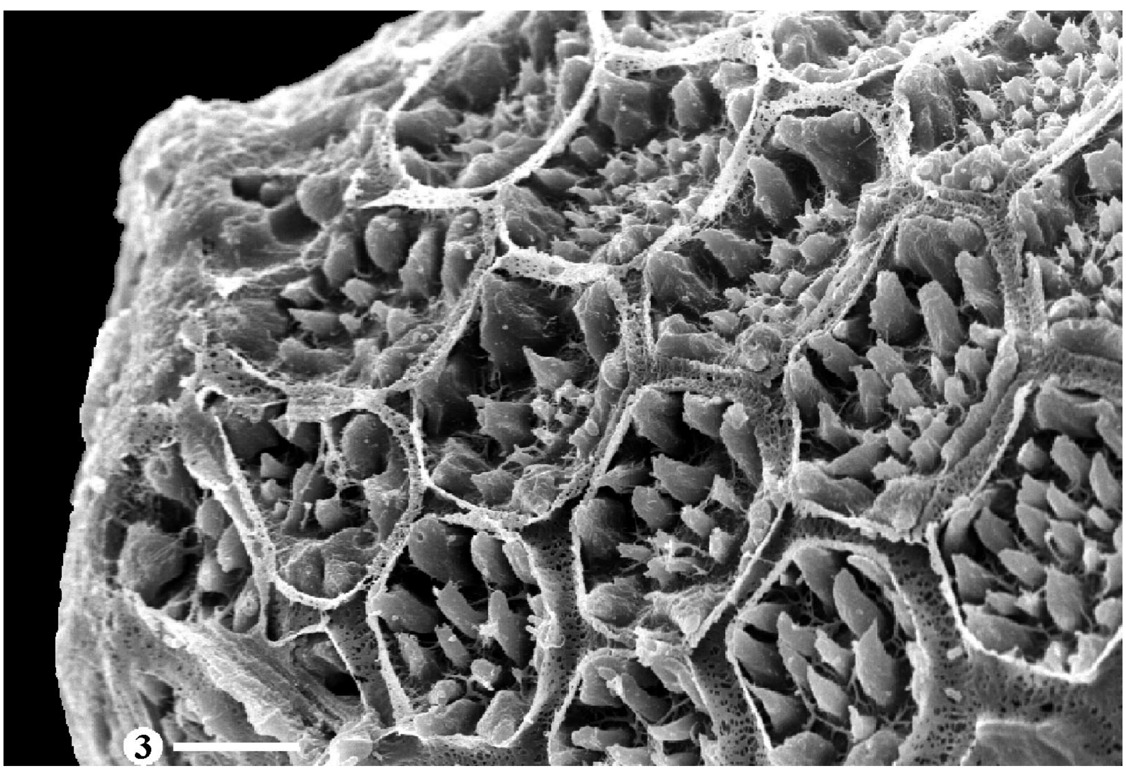
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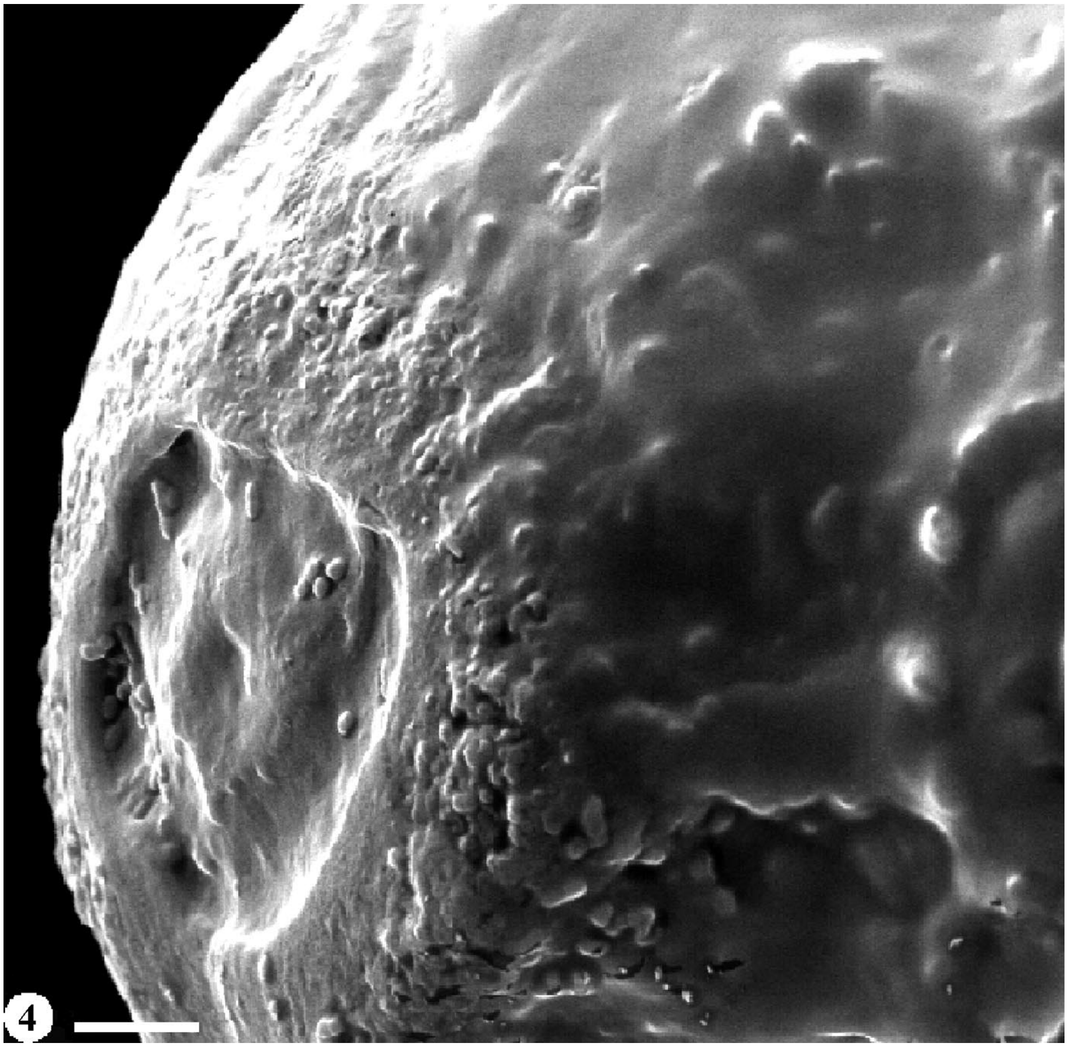

Fig. 4. Micropylar apparatus with a poorly visible frame of the collar.

egg by effectively increasing the adhesion surface area at the time of oviposition.

In our study, we did not observe the presence of filaments in the eggs of *Oc. terrens,* although the presence of elongated fusiform tubercles on the dorsal surface of the eggs suggests that these structures facilitate the adhesion of the eggs to the oviposition substrate, because such structures are not observed on the ventral surface, where the tubercles are small, rounded, and of smooth appearance. It should be noted that female *Oc. terrens, Hg. equinus,* and *Hg. janthinomys* all oviposited in small-sized habitats such as tree holes, including both artificial and natural breeding sites. Firm adhesion seems to protect these eggs from removal by predators and heavy rainfall (Lee et al. 1982, Linley et al. 1991).

Matsuo et al. (1974) studied the eggs of *Aedes* species and described chorionic cells with similar morphology to that found for *Oc. terrens,* thus corroborating the taxonomic closeness between these species.

The importance of obtaining and systematically recording such data can be demonstrated through the possibility of constructing taxonomic keys for separating the species involved, as done by Kalpage and Brust (1968) in providing a key for the eggs of *Aedes* from Manitoba, Canada.

In the present study, similar patterns of distribution of chorionic cells and tubercles were observed when *Oc. terrens* and *Aedes* (*Finlaya*) *albolateralis* Theobald were compared (Matsuo et al. 1974). On the other hand, the presence of a central papilla, which has been described in several species of Aedenii, such as *Aedes aegypti* Linnaeus, *Ae. albopictus* Skuse, *Ae. pseudoalbopictus* Borel, and *Ae. alcasidi* Huang (Matsuo et al. 1974), was not found in the specimens of *Oc. terrens* that we stud-

ied. Similar results were reported for *Hg. janthinomys* and *Hg. capricornii* Lutz (Alencar et al. 2003, 2004).

In conclusion, with full knowledge of the morphology of the eggs of each species, it will be possible to develop keys for the identification of mosquito eggs collected during ovitrapping programs and thus reduce costs and time required for hatching and rearing immature stages to adults for identification.

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